DATA EVALUATION RECORD
HONEY BEE - FIELD TESTING FOR POLLINATORS

ï 141-5 (OPPTS 850. 3040)

1. CHEMICAL: Clothianidin PC Code No.: 044309

2. TEST MATERIAL: Clothianidin FS 600B G Purity: 595 g/L

3. CITATION:

Author: Liepold, K.

Title: Monitoring of potential effects of the drilling of clothianidin treated maize seeds on honeybees, guttation monitoring of maize seedlings under agronomic use conditions and assessment of the relevance of guttation for honeybees in Alsace (France).

Study Completion Date: January 6, 2010

Laboratory: Eurofins-GAB GmbH, Niefern, Oschelbronn, Germany

Sponsor: Bayer CropScience AG, Ecotoxicology, Monheim, Germany

Laboratory Report ID: S09-01402

DP Barcode: 374484

MRID No.: 47972304

4. REVIEWED BY: Moncie Wright, Staff Scientist, Cambridge Environmental, Inc.

Signature: Moncie V Wright **Date:** 08/01/11

APPROVED BY: Teri S. Myers, Ph.D., Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers **Date:** 08/01/11

5. APPROVED BY: Allen Vaughan, Biologist, ERB - V

Signature: **Date:**

6. DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the long-term toxicity of a pesticide to honey bees following an actual-use field exposure. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS:

Scientific Name of Test Organism:	*Apis mellifera* L.
Age or Size of Test Organism at Test Initiation:	Queens in all colonies were of the same lineage and the bees in all colonies were young.
Definitive Study Duration:	122 days (9 day pre-exposure period before a 69-day exposure period followed by a 44-day post-exposure period).

8. CONCLUSIONS:

In a 122 day study (9 day pre-exposure period before a 69-day exposure period followed by a 44-day post-exposure period), the toxicity of dust from clothianidin-treated seed during drilling of treated maize seeds was examined in the honey bee, *Apis mellifera* L., under open field conditions at two test sites (the treatment plots were located in Struth and the control plots in Puberg) in the Alsace region of France. The treated site was planted with maize seeds dressed with the end-use product Clothianidin FS 600B G (AI: 595 g/L Clothianidin), and the other site was planted with untreated control seed. The treatment and the control plots were separated by at least 4 km. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha. Six honeybee colonies were placed at the edge of the each field plot at a distance of 1-2 m from the sowing area with the entrance facing the maize field. The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 19 days before drilling and remained at the study location for 62-63 days after seedling emergence. For the post exposure period the colonies were moved from the exposure plots to a monitoring location near Wissembourg, Alsace, France. Throughout the study, colonies were assessed for mortality, colony strength, and brood and food store area. Additionally, the occurrence and duration of guttation, flight activity and bee behavior, and bees collecting guttation liquid were also observed.

The proportion of guttating plants varied from 0 to 100% of all plants in the respective assessed areas in both the control and treatment plots. The occurrence of guttation was more pronounced in the treatment plot compared to the control plot. During the assessment of guttation in the control plot, no bees were observed on maize plants drinking or collecting water from guttation droplets or dew in the assessment zones. No honeybees were observed consuming guttation liquid in the control or treatment plot for the entire duration of the study period. Further, no behavioral anomalies were observed. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in both the treated and control plots.
Small differences between treatment and control hive data were noted on various dates, but no overall differences in colony strength, mortality, and brood and food area were reported by the study author throughout the study. However, statistically significant differences might have been present between the control and treatment group when comparing the mean mortality data for the entire exposure period (59 days). Further, the reviewer's assessment of brood and food cells yielded possible biologically-relevant differences for the first month after drilling was performed, though statistically-significant differences were not detected due to high variability.

It was concluded that no adverse effects of the potential exposure of honeybee colonies to dust generated during drilling of treated maize seeds and to guttating maize on colony health and development was observed during exposure and in the 43 day post exposure monitoring phase.

Further, no obvious treatment related differences were observed between control and treatment group mortality during exposure.

The reviewer concludes that the data presented in this study are inadequate to accurately determine the effects of clothianidin-treated maize seedlings on honeybees and colony health. Guttation fluid, dead honeybees and pollen and nectar from combs were not analyzed because the study authors determined there was no damage to individual bees or bee colonies due to clothianidin-treated maize exposure.

This study is scientifically sound and *satisfies/does not satisfy* the EFED concerning the guideline requirements for a field toxicity test with honeybees (Subdivision L, ï 141-5 or 850.3040).

9. ADEQUACY OF THE STUDY:

A. Classification: Acceptable / Supplemental / Unacceptable

B. Rationale: N/A

C. Repairability: N/A

10. GUIDELINE DEVIATIONS: There were no guideline deviations.

11. SUBMISSION PURPOSE: This study was submitted to provide data on the toxicity of clothianidin to honeybees in a field test for the purpose of chemical reregistration.

Specifically, the test was conducted to determine the relevance of potentially occurring guttation in young maize plants in the Alsace region in France as a water source for honeybees, and to assess potential effects of Clothianidin residues from the seed treatment of the maize seeds in guttation liquid on bee colonies under field conditions. Additionally, assessments were performed on the potential effects of the maize drilling process during which the colonies might be exposed to Clothianidin-containing dust from the seed treatment.

12. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: **Species of concern (*Apis mellifera*, *Megachile rotundata*, or *Nomia melanderi*)**	*Apis mellifera* L. (Hymenoptera, Apidae)
Colony description at beginning of test:	Each colony occupied hives consisting of two boxes (lower box=brood chamber=1; upper box=honeycomb box=2) that included 10 combs each. Queens in all colonies were of the same lineage and approximately the same age. A queen excluder was placed between the brood chamber and honeycomb box to retain the queen in the brood chamber. There was 1 queen per colony and between 3,693 and 13,323 bees per colony at study initiation.
Pre-test health:	Bees were reportedly free of *Nosema* and *Varroa* disease symptoms.
Supplier	The colonies were supplied by a beekeeper, Mr. Metz, Buhl, Germany
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Exposure Site Location and Establishment:	The test fields were located near La Petite Pierre in the Alsace region of France. The treatment plots were located in Struth and the control plots in Puberg. The treated site was planted with clothianidin-dressed maize seed and the other planted with untreated control seed, giving a total of eight fields. The treatment and the control plots

Guideline Criteria	Reported Information
	were separated by at least 4 km. The size of the field plots was *ca*. 1.8 for the treated plot and *ca*. 2.8 ha for the control. The maize seeds were sown at a nominal drilling rate of 2 units (100,000 seeds)/ha on May 19, 2009. Effective rates: Control: 99,300 seeds/ha Treatment: 103,100 seeds/ha
Site Preparation:	None reported.
Number of Replicates/Treatment:	Six colonies per field plot, with 1 treated and 1 control field plot
Post-exposure Site Location:	Near Wissembourg, Alsace, France.
Lighting:	Natural; not further described.
Precipitation:	Precipitation measured during mortality assessments at the control plot ranged from 0.0 to 22 L/m^2 during the exposure period (data obtained from Figure 23). The maximum rainfall event occurred on *ca*. September 7 and 15, 2009 when 40 and 31 L/m^2 precipitation occurred, respectively.
Temperature:	Daily temperatures ranged from 2.5 to 31.7°C during the exposure period.
Relative humidity:	Mean relative humidity ranged from 50.4 to 97.2% during the exposure period.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported
Reference toxicant tested?	No

Guideline Criteria	Reported Information
Duration of Exposure Period	69 days
Duration of Post-exposure Period	44 days in the monitoring site
Test Substance(s):	Clothianidin FS 600B G Formulation Type: suspension Batch No.: PF90191228 AI: 595 g/L Clothianidin (analyzed)
Control Substance(s):	N/A- control seeds were not treated
Maize Seed:	Seed variety: Ronaldinio
Application Rate:	0.502 mg ai per seed (analyzed)
Verification of Application Rate:	Not reported
Method of Seed Coating:	Not reported
Colony Introduction:	The colonies were placed at the edge of the each field plot at a distance of 1-2 m from the sowing area with the entrance facing the maize field. The colonies were established in a downwind position relative to the field in order to maximize potential dust exposure during drilling. The colonies were placed in the fields 19 days before drilling and remained at the study location for 62-63 days after seedling emergence.

Guideline Criteria	Reported Information
Post-exposure:	The colonies were moved from the exposure plots to a monitoring location near Wissembourg, Alsace, France.
Assessment scheme:	The part of the field plots that was considered to be most likely to be attractive to honeybees seeking water was assessed regarding the occurrence of guttation and/or dew (assessment area). The in-field assessment area (zones 1-4) covered a width of 5 m to the left and to the right from the outer bee hives at each field, and in length encompassed 58 parallel rows of maize (42.75 m). Each assessment started with zone 0 and ended with zone 4.
Assessment zones:	Zone 0 = off-field assessment area; between row number 1; 2-4 m away from the field. Zone 1 = rows 1-7; assessments were performed along each row; observers made assessments while walking. Zone 2 = rows 8-13; assessments were made for rows in groups of 3 (each 3rd row was a passing row). Zone 3 = rows 14-28; assessments were made for rows in groups of 5 (each 5th row was a passing row). Zone 4 = rows 29-58; assessments were made for rows in groups of 5 (each 10th row was a passing row). Additionally, there were six 2 m^2 plots that each covered 2 rows of maize seedlings.

D. Biological Assessments

Guideline Criteria	Reported Information
Maize guttation:	The proportion of maize plants displaying guttation and/or dew was monitored for a

Guideline Criteria	Reported Information
	maximum of 53 days, until no more guttation could be seen over 6-7 days. This was determined by observers that walked through each passage row. The percentage was estimated at 10, 25, 50, 75, 90, and >90%. If less than 10% of the plants displayed guttation, the exact number of plants in an assessment row that showed guttation was counted. Guttation occurrence was checked in regular intervals from the early morning onwards until no more guttation droplets were visible. In addition, the general occurrence of guttation droplets was checked at each field at sunset of every day. One full observation period included the guttation assessments in the 4 established zones in the fields. Additionally, zone 0 was checked for the presence of guttation and/or dew on the off-field vegetation and to determine if the extent of guttation and/or dew on the off-field vegetation was more or less than that present on the plants in the maize field. If no guttation occurred at both field sites then the plants of neighboring fields or adjacent vegetation were checked for guttation.
Bees collecting guttation droplets:	After the assessment of guttation and honeybee activity in the zones the number of honeybees per assessment plot sitting on the ground or on plants, and the number taking up droplets was recorded during a 4 minute assessment period per plot. Any abnormal behavior was documented.
Flight activity:	On each assessment day (those days on which guttation was observed), the flight activity at the hive entrance of each hive was documented

Guideline Criteria	Reported Information
	at the start and end of each observation period. Flight activity was assessed by counting the number of bees entering the hive over 1 minute and by counting the number leaving the hive over 1 minute.
Mortality:	Linen sheets were spread on the ground in front of the hives and dead bee traps were attached to the entrance of each hive to measure mortality during the exposure period. Mortality was assessed nine days before drilling, on the day of seeding (after seeding was done), and daily thereafter until the termination of the exposure phase. The dead bee traps were emptied daily at the same time of day and the bees were transferred within 10 hours into a deep freezer (≤-18°C) for potential residues analysis.

Guideline Criteria	Reported Information
Colony condition:	The condition of the colonies was recorded once before the hives were placed on the field plots and afterwards in weekly intervals during the exposure phase.
Brood:	During the monitoring phase the brood assessments were performed 7 times in weekly intervals. The following parameters were assessed: - Colony strength (number of bees) - Presence of a healthy queen (presence of eggs) - Pollen storage area and area with nectar or honey - Area containing cells with eggs, larvae, and capped cells The comb area covered with bees and cells with nectar, pollen, egg, larval, and capped cells was estimated per comb side and the total number of bees and cells containing the brood stages, pollen, and nectar on the comb was calculated. The mean values were calculated for each hive and assessment date.
Collection of guttation fluid:	Guttation fluid was sampled on days when sufficient guttation for sampling was available early in the morning in the treated plot. The samples were collected in the morning within the first hour of the assessments on the field outside the guttation assessment areas and in a distance of at least 20 m from the hives. The fluid was collected with plastic Pasteur pipettes and was stored in Eppendorf caps. Samples were stored on blue ice and transferred within 14 hours to a deep freezer (≤-18°C). During the trial, sampling occurred on 29 days.

Guideline Criteria	Reported Information
Collection of pollen and nectar from combs:	Samples of pollen and nectar were collected from the bee hive combs during each brood assessment after drilling during the exposure phase. If possible, one sample that weighed 1 gram was taken per colony in the control and treated plots. Each sample was taken from 3 different sections per hive, and then all 3 samples were pooled. Pieces of comb were cut from the comb using a clean knife for each sample. A spoon was used to collect nectar. Samples were stored cooled and transferred within 10 hours to a deep freezer (≤-18°C). No further preparation was performed because the residues were not analyzed.

E. Residue Analysis

Guideline Criteria	Reported Information
Guttation fluid, dead bees, pollen and nectar from combs:	The study author concluded that Clothianidin-treated maize did not have negative effects on any of the biological endpoints measured; therefore, the author deemed it unnecessary to perform residue analysis.

13. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Signed and dated No Data Confidentiality, GLP, and Quality Assurance Statements were provided. This study was conducted in compliance with the most recent edition of the Principles of Good Laboratory Practice, Chemikaliengesetz, Attachment 1, Germany, and the OECD Principles of Good Laboratory Practice.

Guideline Criteria	Reported Information
	This study was not conducted according to any established guidelines; therefore, it was performed according to the study plan and SOPs of eurofins-GAB.
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Observations of guttation and proportion of guttating plants:
Guttation was observed in the morning and evening. Guttation on adjacent vegetation and on neighboring fields was observed on most days when guttation occurred on the treatment maize plot. Guttation was observed on a few days on adjacent vegetation toward the end of the assessment period in the control plot.
The proportion of guttating plants varied from 0 to 100% of all plants in the respective assessed areas in both the control and treatment plots. The occurrence of guttation was more pronounced in the treatment plot compared to the control plot. Dew and guttation did not occur together on all assessment days. Generally, there were more days with occurrences of guttation only as compared to days with both guttation and dew.

Honeybees visiting plants displaying guttation:
During the assessment of guttation in the control plot, no bees were observed on maize plants drinking or collecting water from guttation droplets or dew in the assessment zones. During 4 assessments out of 147 in the control plot, 1-2 bees were sitting on a maize plant, 1 was sitting in the field on the ground, and 1 bee was flying around plants with dew. In the 2 m^2 observation areas, bees were found sitting on the ground or on plants on 5 out of 97 assessments, but not interacting with guttation droplets.

In the treated plot, 1 or 2 single bees per assessment were observed sitting on plants or on the ground or flying over the crop in 7 of the 133 assessments. In the 2 m^2 areas, bees were on the ground or on plants for 2 out of 74 assessments (one single bee per area), but not interacting with guttation droplets.

No honeybees were observed consuming guttation liquid in the control or treatment plot for the entire duration of the study period.

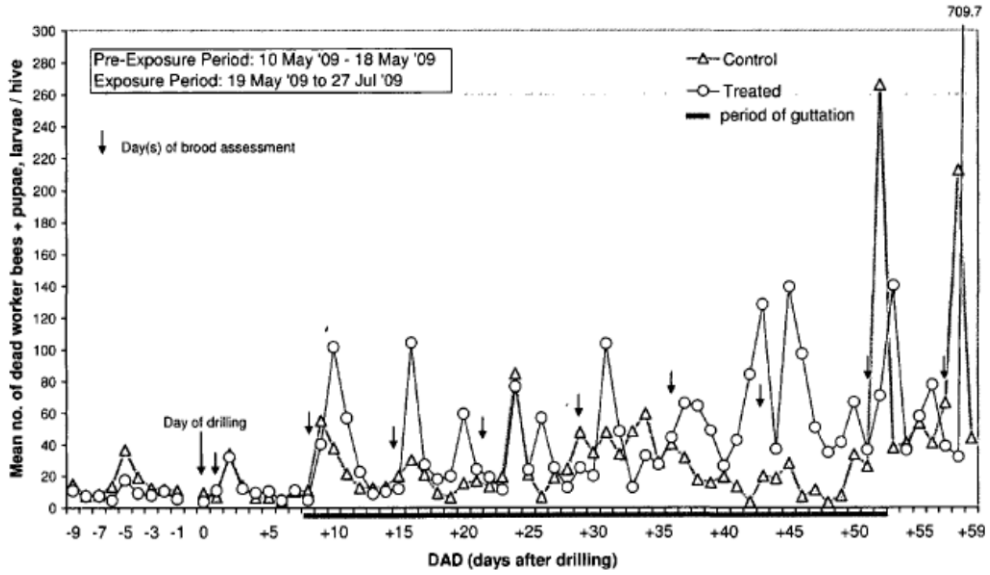
Flight activity:

Flight activity was low in the morning due to low temperatures. Flight activity increased during the course of the study in both plots. The period of guttation and bee activity overlapped. Bee behavior in the front of the hives was normal in the both the treated and control plots. Intensive flight activity was observed for 17 assessment days in the treated plot and for 3 days in the control plot. No behavioral anomalies were observed.

Mortality:

The daily mean pre-exposure (days -9 to -1) mortality (linen sheets + dead bee traps) in the control and treatment groups was 15 and 9 bees/hive, respectively. On the day of drilling (but after the process was complete), mortalities averaged 9.2 dead bees/hive in the control field as compared to 3.8 dead bees/hive in the treated field. One day after drilling, control mortality averaged 6.8 dead bees/hive and treatment mortality averaged 10.7 dead bees/hive. An increase in mortality was observed on the second day after drilling (DAD+2) in both the control and treatment group; however, the study author attributed this to the brood assessment that was carried out the day before. For the remaining assessment days, mean daily mortality of both the control and treatment groups fluctuated (Figure 1). Conspicuous peaks of mortality occurred in both the control and treatment group. However, peaks in treatment mortality were more frequent and the difference between the treatment and control mortalities was much greater. Increases in the number of dead bees in front of the hives were mainly observed after the brood assessments that were performed during exposure in both treatment groups. Mortality peaks were frequently caused by only one or a few of the individual colonies of the respective treatment group. From 37 days after drilling until exposure phase termination, mortality was slightly increased in the treated colonies, which led to a difference in mortality in front of the hives from day 0 to day 59 of 32 dead bees/hive in the control as compared to 54.5 dead bees/hive in the treatment group. The mean daily mortality during guttation (days 8 to 53 after drilling) was 29.8 and 46.3 dead bees/hive in the control and treated groups, respectively. The mean daily mortality for the entire exposure (59 days) was 32 and 54.5 bees/hive in the control and treated groups, respectively. However, the study author concluded that mortality in the control and treated groups was low and comparable, and that there was no treatment-related effect. The strong peaks in mortality in the control and treatment groups on days 52 and 59 after drilling were due to brood assessments performed the days before. The high mortality was attributed to robbery, as there was an abundance of nectar in the hives.

Figure 1. *Mean number of dead worker bees, pupae, and larvae/hive/day collected in the dead bee traps and on the linen sheet in front of the hives in the control and treatment groups before and during the time of exposure at the test site.*



Colony condition and brood development:

At the first brood assessment, colony strength (=mean number of bees/hive) in the control hives ranged from 6,880 to 13,323 bees. Colony strength in the treatment hives ranged from 3,693 to 12,881 bees. Only the bees that were present in the hives at the time of the assessment were included in the estimates. A portion of the worker bees was outside foraging, so the estimates underestimate actual colony strength. Mean colony strength was similar at the first brood assessment, and was followed by an increase in both the control and treatment group. Colony strength was similar in subsequent assessments. At the 5th brood assessment, mean colony strength in both groups decreased until the 10th assessment where the lowest values of the study period were observed (Figure 6). Until the 11th assessment, the development of colony strength was comparable. At the 12th assessment, mean strength of the treated colonies increased to the highest levels while the mean strength of the control colonies remained lower. Between the 14th and 15th assessments the mean colony strength of both the control and treatment group decreased and then increased at the last 2 assessments. The low mean strength of the control colonies was mainly influenced by three colonies (C4, 5, and 6), which were weak at the 9th assessment until the end of the study. At the 16th assessment, hive C4 was dead, and at the 17th assessment, hive C5 was dead. One colony (T2) of the treated group was dead at the 13th brood assessment. The exclusion of the dead colonies for further calculations of mean values led to an increase in mean values of the respective treatment group. The remaining colonies of the control and treatment group were of equal strength or stronger as compared to the first assessment.

The development of the mean brood area size on the combs (eggs, larvae, and pupae) in the control and treatment group was similar. Only slight differences were observed between the mean amount of food stores in the combs (nectar and pollen) in the control and treatment group (Figures 3 and 4). Lack of pollen was only observed in the treatment group in hives T4 and T6 on June 10, 2009. Lack of nectar was only observed in the control group in hive C4 on August 26, 2009 and in hive C6 on September 2, 2009.

Figure 2. *Mean number of honeybees per hive (=colony strength) in the control and treatment group.*

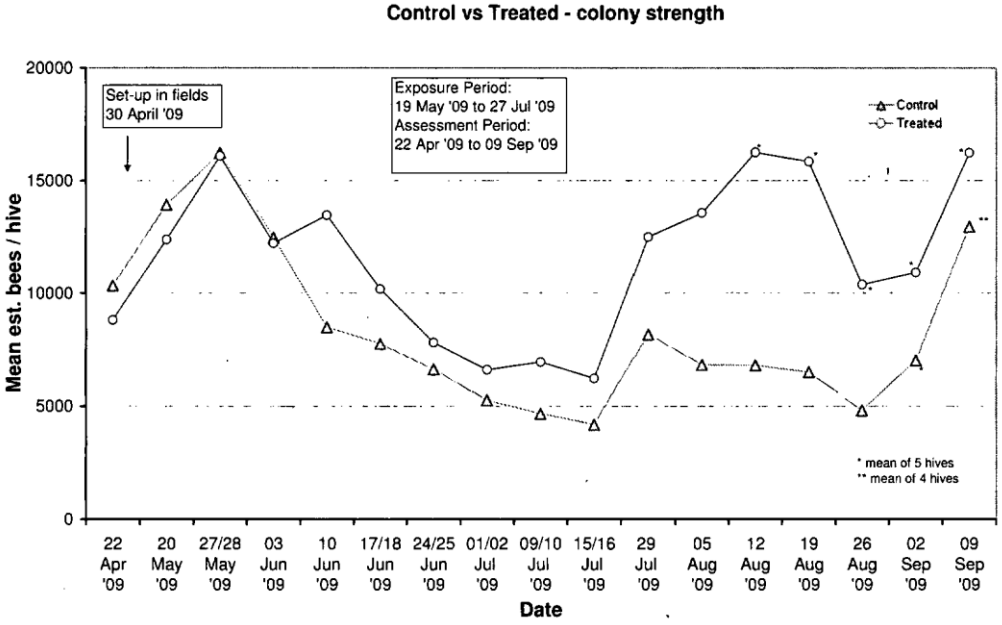


Figure 3. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the treatment group.*

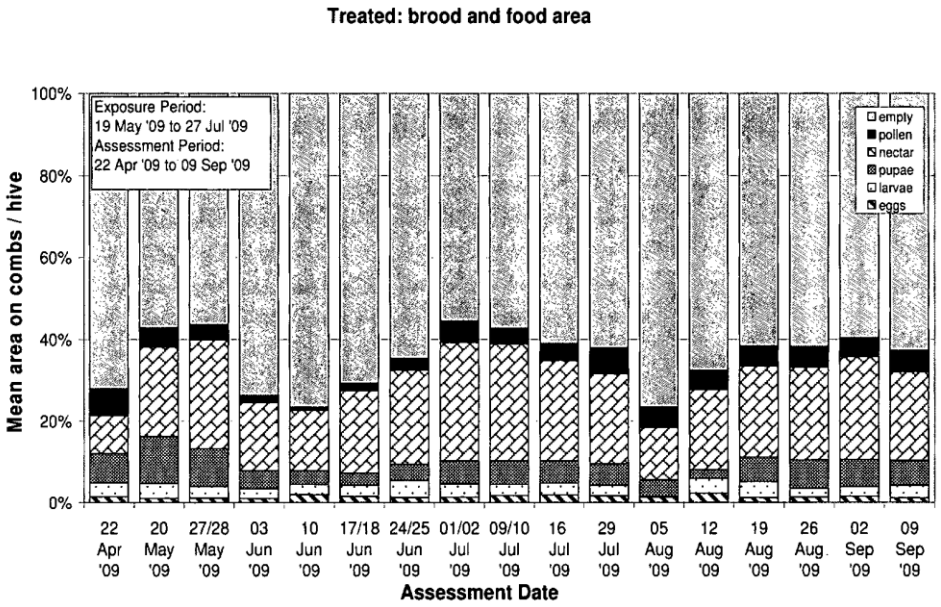
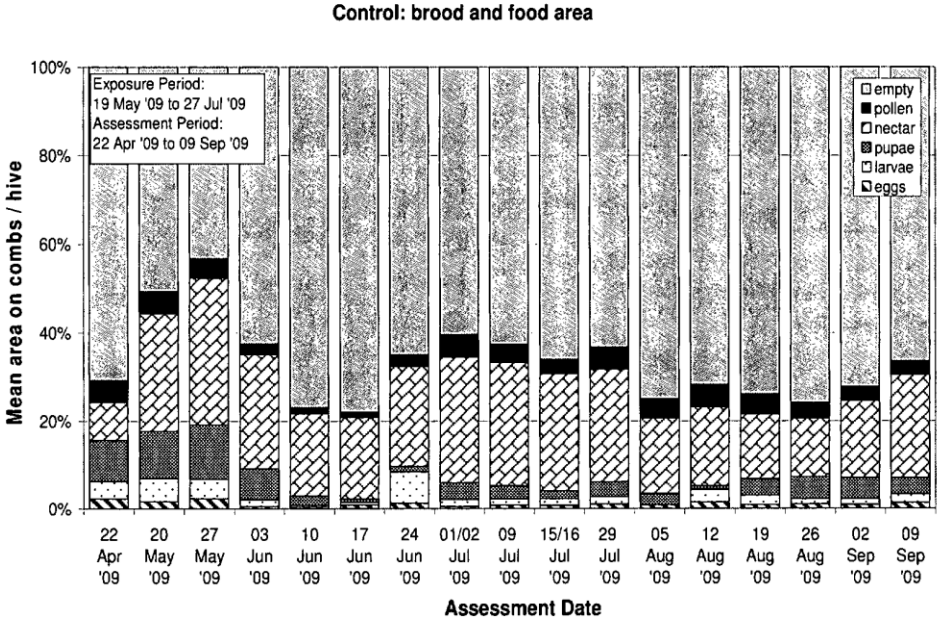


Figure 4. *Mean comb area per hive (%) covered with brood cells (eggs, larvae, and pupae) and with food stores (nectar and pollen) in the control group.*



Reported Statistical Results:

The study author did not perform statistical analysis on any of the parameters measured.

14. REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:

Replicate data were provided for the bee trap mortality data when considering each individual hive as a replicate. However, individual hive data was not provided for the mortality data obtained from linen sheets placed in front of each hive. There were high average mortalities recorded on the linen sheets of the treatment group for both the exposure during guttation period (8 to 52/53 DAD) and the entire exposure period (0-59 DAD). For the 8-53 DAD period, mortalities averaged 34 bees/hive in the control and 152 bees/hive in the treatment group. For the 0-59 DAD total exposure period, mortalities averaged 38 bees/hive in the control and 162 bees/hive in the treatment group. If individual hive linen sheet mortality data had been reported, the reviewer would likely have been able to determine statistically significant differences between the control and treatment group based on linen hive mortality only. When considering total mortality (bee traps + linen sheets), statistically significant differences might have been detected between the control and treatment group (control = 32 bees/hive vs treatment = 55 bees/hive).

The reviewer visually verified the reported results and agrees with the study author's assessments with regard to colony strength. The colony strength in the treatment group was higher than that of the control group for all assessment days except the first day assessments were made after drilling when the control had higher colony strength.

The reviewer visually assessed the brood and food area data and conducted statistics when the treatment values were much lower than the control values. First, the pre-assessment data was examined for differences between the control and treatment groups. All parameters were very similar or the treatment group had higher values than did the control, with the exception of egg and pupal cells. The sum area of egg cells averaged 4367 in the control as compared to 2867 in the treated group. The sum area of pupal cells averaged 18,000 in the control as compared to 13,800 in the treated group. The reviewer compared the control and treatment groups using a two-tailed t-test assuming equal variances in Excel 2003 to determine if there were already significant differences present before drilling was initiated. No significant differences were detected.

The reviewer visually assessed all data collected after drilling, and determined that there were possible significant differences present for select parameters within *ca*. 1 month of drilling. For all other assessments after June 24, the control and treatment data were comparable and any differences observed appeared to be attributed to natural variability that is characteristic of field studies. For many of the assessment parameters, the treated group had higher brood and food

values than did the control group. For 1 and 8-9 days after drilling (DAD), the sum areas of larval and nectar cells appeared to differ significantly between the control and treated groups. For 15 and 22 DAD, the sum areas of nectar and pollen appeared to be significantly different between the control and treated groups. At 36-37 DAD, the sum area of larval cells appeared to be significantly different. The reviewer compared the control and treated groups for these assessments/parameters using a two-tailed t-test assuming equal variances. High variability present in both groups precluded the ability of the tests to detect significance.

The remaining parameters for brood and food area were very similar when comparing the control and treatment groups.

Summary of parameters statistically analyzed by the reviewer.

Assessment day	**Treatment**	**Sum area of egg cells**	**p-value**	**Sum area of pupal cells**	**p-value**
-27 DAD	Control	4367	0.14	18,000	0.29
	0.502 mg ai/seed	2867		13,800	
		Sum area of larval cells	**p-value**	**Sum area of nectar cells**	**p-value**
1 DAD	Control	10,167	0.16	51,433	0.28
	0.502 mg ai/seed	7,033		42,167	
8-9 DAD	Control	8,533	0.34	63,900	0.15
	0.502 mg ai/seed	5,533		51,233	
		Sum area of nectar cells	**p-value**	**Sum area of pollen cells**	**p-value**
15 DAD	Control	49,800	0.064	4,500	0.39
	0.502 mg ai/seed	31,867		3,567	
22 DAD	Control	36,000	0.29	2,733	0.28
	0.502 mg ai/seed	28,400		1,400	
36-37 DAD		**Sum area of larval cells**	**p-value**		
	Control	13,867	0.40		
	0.502 mg ai/seed	8,100			

Differences between control and treatment were present suggesting the possibility of treatment-related effects for some time periods; however, the high variability among replicates precluded detection of any effects, if they existed.

16. REVIEWER'S COMMENTS:

The reviewer's conclusions mostly agreed with the study author's; however, the reviewer believes that statistically significant differences might have been detected between the control and treatment group when comparing the mean mortality data for the entire exposure period (59 days). Further, the reviewer's assessment of brood and food cells yielded possible biological significance for the first month after drilling was performed, though statistical significance could

not be determined due to high variability. Small differences between treatment and control hive data were found on various dates, but there were essentially no differences in colony strength throughout the study. There was high variability present in this study that precluded the ability of the t-tests to indicate statistical significance. As a result, there are limitations on the both the results and the reviewer's ability to determine if there was in fact a treatment related effect of clothianin-dressed maize seed on honeybees.

Climatic data (temperature, humidity, rainfall, and cloud formation) were recorded at the control field plot. Temperature and humidity were recorded at 15 minute intervals using a data logger starting May 27, 2009. Daily rainfall was measured using a rain gauge. Data from May 1 to May 26, 2009 were taken from an official weather station in Berg. While colonies were located at the monitoring location, weather data were collected from the nearby official government weather station in Hegeney.

Soil samples were collected from the test fields for determination of physico-chemical properties. Five soil cores (5 cm width) were collected to a depth of 20 cm from each corner of the treated and control field plot (4 x 5 samples per field). Standard soil parameters were determined:

	Control	Treatment
Soil Type[6)]	Medium silty sand	High silty sand
pH value ($CaCl_2$) [1)]	5.0	4.8
WHC_{max} [g /100 g soil dry weight] [2)]	50.3	51.3
CEC [mval Ba/ 100 g soil dry weight] [4)]	9.5	11.3
TOC [%] [3)]	1.59	2.04
Clay [%] (< 0.002 mm) [5)]	2.7	3.1
Silt [%] (0.063 mm to ≥ 0.002 mm) [5)]	32.7	50.0
Sand [%] (2 mm to ≥ 0.063 mm) [5)]	64.7	46.9

WHC_{max} = Maximum Water Holding Capacity
CEC = Cation Exchange Capacity
TOC = Total Organic Carbon
[1)]DIN ISO 10390 mod.
[2)]Schaller 1993
[3)]DIN ISO 10694
[4)]Mehlich method mod.
[5)]DIN 19683
[6)]DIN 4220

16. REFERENCES:

DIN 19683 BLATT 3 (1973-04): Physikalische Laboruntersuchungen – Bestimmung der Korngrossenzusammensetzung.

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OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION

27 days before drilling egg cell sum area	*Control*	*0.502 mg ai/seed*
Mean	4366.666667	2866.666667
Variance	1782666.667	3466666.667
Observations	6	6
Pooled Variance	2624666.667	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.603669275	
P(T<=t) one-tail	0.069934348	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.139868696	
t Critical two-tail	2.228138842	

27 days before drilling pupal cell sum area	*Control*	*0.502 mg ai/seed*
Mean	18000	13800
Variance	40480000	45568000
Observations	6	6
Pooled Variance	43024000	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.10905868	
P(T<=t) one-tail	0.146681959	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.293363919	
t Critical two-tail	2.228138842	

1 DAD larval cell sum area	*Control*	*0.502 mg ai/seed*
Mean	10166.66667	7033.333333
Variance	11414666.67	14358666.67
Observations	6	6
Pooled Variance	12886666.67	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.511809008	
P(T<=t) one-tail	0.080760592	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.161521185	
t Critical two-tail	2.228138842	

1 DAD nectar cell sum area	*Control*	*0.502 mg ai/seed*
Mean	51433.33333	42166.66667
Variance	50614666.67	336598666.7
Observations	6	6
Pooled Variance	193606666.7	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.153517041	
P(T<=t) one-tail	0.137761642	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.275523284	
t Critical two-tail	2.228138842	

8-9 DAD larval cell sum area	*Control*	*0.502 mg ai/seed*
Mean	8533.333333	5533.333333
Variance	19690666.67	34378666.67
Observations	6	6
Pooled Variance	27034666.67	
Hypothesized Mean Difference	0	
df	10	
t Stat	0.999358642	
P(T<=t) one-tail	0.170594358	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.341188716	
t Critical two-tail	2.228138842	

8-9 DAD nectar cell sum area	*Control*	*0.502 mg ai/seed*
Mean	63900	51233.33333
Variance	140428000	247350666.7
Observations	6	6
Pooled Variance	193889333.3	
Hypothesized Mean Difference	0	
df	10	
t Stat	1.575600139	
P(T<=t) one-tail	0.073097886	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.146195771	
t Critical two-tail	2.228138842	

15 DAD nectar cell sum area	*Control*	*0.502 mg ai/seed*
Mean	49800	31866.66667
Variance	237952000	207898666.7
Observations	6	6
Pooled Variance	222925333.3	
Hypothesized Mean Difference	0	
df	10	
t Stat	2.080376484	
P(T<=t) one-tail	0.032078628	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.064157257	
t Critical two-tail	2.228138842	

15 DAD pollen cell sum area	*Control*	*0.502 mg ai/seed*
Mean	4500	3566.666667
Variance	3276000	3318666.667
Observations	6	6
Pooled Variance	3297333.333	
Hypothesized Mean Difference	0	
df	10	
t Stat	0.89025819	
P(T<=t) one-tail	0.197120488	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.394240976	
t Critical two-tail	2.228138842	

22 DAD nectar cell sum area	*Control*	*0.502 mg ai/seed*
Mean	36000	28400
Variance	142176000	141344000
Observations	6	6
Pooled Variance	141760000	
Hypothesized Mean Differen	0	
df	10	
t Stat	1.105598313	
P(T<=t) one-tail	0.147394677	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.294789355	
t Critical two-tail	2.228138842	

22 DAD pollen cell sum area	*Control*	*0.502 mg ai/seed*
Mean	2733.333333	1400
Variance	6186666.667	1840000
Observations	6	6
Pooled Variance	4013333.333	
Hypothesized Mean Differen	0	
df	10	
t Stat	1.152780835	
P(T<=t) one-tail	0.137905806	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.275811612	
t Critical two-tail	2.228138842	

36-37 DAD larval cell sum area	*Control*	*0.502 mg ai/seed*
Mean	13866.66667	8100
Variance	232906666.7	21596000
Observations	6	6
Pooled Variance	127251333.3	
Hypothesized Mean Difference	0	
df	10	
t Stat	0.885430148	
P(T<=t) one-tail	0.198358165	
t Critical one-tail	1.812461102	
P(T<=t) two-tail	0.39671633	
t Critical two-tail	2.228138842	